FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 15 ISSUE 5 ■ SEPTEMBER–OCTOBER 2007

SIGNAL DISCOVERY OPENS PATHWAY TO THERAPIES FOR IRON-OVERLOAD ILLS

by Christopher Wanjek

Christopher Wanjek

Jeff Miller

Too much iron will leave a body frail, destroying joints, scarring the liver, fueling cancers and heart disease, and sapping vitality. These effects contradict everything we have learned from the nautical sage Popeye.

The human body has no means to shed excess iron aside from blood loss. So it regulates iron's uptake from food through the peptide hepcidin, which hinders iron absorption via enterocyte cells in the gut.

The basics are well known. In the past year, however, Jeff Miller's group in NIDDK's Molecular Medicine Branch has reached a breakthrough in understanding the underlying chemical signaling that controls iron regulation, which could lead to diagnostic tools and possible treatment for those with iron-overload diseases such as thalassemia.

Miller, chief of NIDDK's Molecular Genomics and Therapeutics Section, describes this research in the September 2007 issue of *Nature Medicine*, with lead author Toshihiko Tanno from his lab and other scientists from NIH and elsewhere.[1]

The team speculates that the newly discovered signal, involving growth-differentiation factor 15 (GDF15), might be common in many states of anemia and blood disorders as well as in liver disorders and some cancers.

"While what we were studying was

continued on page 4

A Decade of Building Expertise

NISC: TRACING THE SEQUENCE OF EVENTS LEADING TO 21ST-CENTURY GENOMICS

by Raymond MacDougall

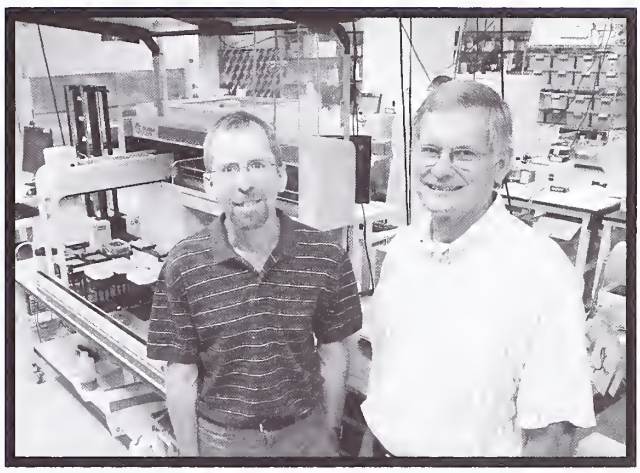
Ten years ago, the NIH Intramural Sequencing Center (NISC) began humbly with six gel-based sequencing machines crowded into a few rooms of borrowed lab space.

Today, NISC is not only one of the jewels of the NIH intramural program but also a highly productive sequencing center at the forefront of contemporary worldwide genomics research.

"NISC now plays a key role in the ongoing NIH effort to unravel the genetic complexities of human health and disease," said NHGRI Director Francis Collins. "It has served as a vital component of numerous important sequencing projects over the past 10 years, all aimed at achieving an understanding of the human genome."

NISC researchers have also played a key role in large-scale collaborative projects, such as the ENCyclopedia Of DNA Elements (ENCODE; see *The NIH Catalyst*, September-October 2005, page 1).

A focus of NISC's current research portfolio is comparative sequencing—sequencing and studying the genomes of other vertebrate species. The ENCODE project has capitalized on this expertise; NISC has to date been credited with sequencing a targeted 1 percent of the genomes of 26 mammals, from the hedgehog to the elephant.



Maggie Bartlett

At home in a room with a view into multiple genomes: *Eric Green (left), NHGRI scientific director and NISC director, and Robert Blakesley, associate investigator in the NHGRI Genomic Technology Branch and head of the NISC Sequencing Group*

Progressions

NISC's original mandate in 1997 was to provide NIH intramural investigators

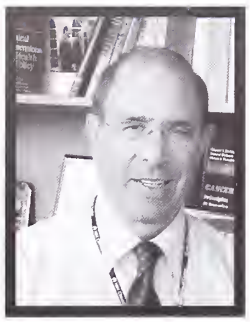
continued on page 6

Research Gala

The 20th NIH Research Festival, September 25–28, *2007, held at Masur and Natcher, presents cutting-edge research: plenary session, 21 symposia, posters galore—with job fair, music, and food to boot. For details, visit <http://researchfestival.nih.gov>.*

CONTENTS

A Space Odyssey: Regarding the Need to Utilize Unused Capacity at the Clinical Center



John Gallin

If you'd appreciate a lively discussion with colleagues, ask how the NIH Clinical Center can best be used. That's a topic that has generated interesting and thoughtful discussions since the original hospital opened in 1953. And now, with the opening of the new Mark O. Hatfield Clinical Research Center just over two years ago, these discussions are as energetic as ever.

Indeed, on July 12, 2007, the NIH institute directors held a mini-retreat to contemplate a new direction for the Clinical Center. During that meeting, I presented a vision to initiate cross-institute intramural activities as well as new partnerships with the extramural academic community and industry, ideas aimed at infusing new intellectual vigor while capitalizing on unused resources at the Clinical Center. The ideas I presented need and are getting further discussion and vetting, including a joint meeting of the scientific directors and clinical directors in early September at which exciting long- and short-range topics were endorsed for continued development.

Who and How Many Are the Clinical PIs ?

There has always been unused capacity at the Clinical Center. Historically, the situation was related to limited resource availability in the intramural programs. More recently, however, the reasons have become more complicated and driven by a convergence of factors, including declines in funding availability and in the number of senior investigators writing research protocols.

The number of talented principal investigators (PIs) writing clinical protocols is perhaps the most important driver of patient activity and the quality of clinical investigations.

Although the absolute number of PIs writing clinical research protocols has increased—from 461 in 2001 to 547 today—both the absolute numbers and the percentages of clinical protocol writers among the total tenured and tenure-track investigator pools have declined.

In 2001, the 192 tenured senior investigators writing clinical protocols represented 21 percent of the total pool of senior investigators at NIH. Today, 156 tenured investigators, representing 16 percent of the pool, are writing such protocols. The decline in clinical PIs among tenure-track investigators is even more dramatic: from 48, or 19 percent, in 2001 to 18, or 7 percent, today. The PIs at NIH to whom credit can go for the absolute increase in clinical research protocols are NIH's staff clinicians.

The decline in the number of tenured and tenure-track scientists writing clinical protocols is worrisome and indicates we have a problem recruiting and nourishing the career paths of young clinical investigators. The Careers in Clinical Research Working Group of the Advisory Board for Clinical Research, chaired by Lynette Nieman, is working actively to provide new opportunities for clinical investigators at NIH.

Difficulties in recruiting and retaining clinical investigators are not unique to the NIH Clinical Center.[1] It is a national problem. NIH Director Elias Zerhouni has championed the need to revitalize clinical research in the United States.[2] The new Clinical and Translational Science Awards led by the National Center for Research Resources were designed to invigorate the national clinical research effort by providing a network of academic homes for clinical research around the nation.

As we contemplate the future of the Clinical Center intramurally, we also need to consider its role in rebuilding the nation's clinical research enterprise. A strong Clinical Center that serves the needs of the intramural program while serving as a resource for the country is an ideal goal.

MEC Suggestions To Pool Resources In Recruiting Clinical Investigators . . .

A top priority of the Medical Executive Committee (MEC) is reversing the falling numbers of tenured and tenure-track clinical investigators who are patient-oriented. Creating improved career paths for clinical investigators is one approach. The MEC has also suggested central recruitment and trans-institute pooling of resources to create exceptional recruitment packages to attract the most promising tenure-track investigators. Our salaries are competitive for young investigators, and recent increases allow for reasonable pay in the subspecialties, but it is this unique ability to draw resources from multiple institutes that can offer an unrivaled opportunity for clinical investigators—and that is what we need to provide.

The expectation is that young tenure-track clinical investigators will develop strong scientific programs with clinically based components. After central recruitment, each investigator would be assigned to the appropriate institute among those that had agreed in advance to participate in the recruitment process.

. . . and in Responding to Public Health Crises

Another way for central planning to attract clinical investigators, the MEC has suggested, is for NIH to identify trans-institute "Manhattan Projects" to address top public health priorities. NIH has gathered such forces in response to such public health crises as the AIDS epidemic in the 1980s and the current obesity epidemic.

But we need additional compelling projects that enlist diverse groups of basic and clinical investigators to find solutions to serious public health problems. The recently established trans-NIH effort in inflammation, autoimmunity, and immunology, led by Neal Young,[3] is one example of a large group effort to pool the talents of investigators to address an area of emphasis from which will evolve projects related to specific diseases.

In addition to large trans-NIH efforts, the MEC recommended that each institute identify at least three areas of emphasis for its intramural programs that articulate the strengths and directions of that institute. The MEC has also called upon NIH to establish a trans-NIH strategic plan for clinical research, something we have never had in the past.

More Suggestions: Reopen Clinics For Undiagnosed Patients with Rare Diseases . . .

Sustaining the intellectual vitality of our clinical programs is essential. One important contributor to the vitality of programs is a diverse population of patients with challenging clinical problems. Such a population of patients helps strengthen the clinical skills of the practicing physicians and provides an environment for generating hypotheses for clinical research. Before the mid-1990s, the Clinical Center operated clinics for patients who presented difficult clinical challenges, or "fascinoma clinics."

These clinics served two major purposes: They offered a beacon of hope for many patients who obtained access to the special clinical expertise at NIH, and they generated intellectual excitement and support for hypothesis generation at the Clinical Center. I recall well the fever-of-unknown-origin clinic created by the late Sheldon Wolff in the 1970s. It benefited patients with previously undiagnosed clinical problems and helped to spawn the field of clinical immunology. There are many other past examples of such rewarding developments throughout the NIH institutes. But this innovative clinic activity was curtailed dramatically in 1994 by an inspector general who believed the clinics were catering to the rich and the famous. We need to reopen these clinics with clearly defined trans-NIH planning, coordination, and strong oversight.

. . . and Open Clinical Center Doors To the Extramural Community

The Clinical Center has special resources that in some cases are unique. Despite the reduction in rare-disease clinics, the cohorts of patients with rare diseases are still substantial. A recent review of our patient population indicated that about 44 percent of our 2006 admissions were patients with a rare disease, and we estimate that we are now actively following 37,500 such patients. Years of studies of patients with rare diseases has led to unequaled phenotyping capability at the Clinical Center. Special resources also include our new metabolic patient unit designed to study obesity, extraordinary imaging capacity, the biomechanics laboratory in our Rehabilitation Medicine Department, and training in clinical research extended to trainees ranging from medical students through senior investigators.[4] These resources can be utilized to a greater extent.

Making these resources available to extramural colleagues in academia and industry will generate new partnerships, collaborations that enrich both the intramural and extramural communities. Strong relationships with the extramural community will also serve the intramural program well politically—we will be viewed not only as a beacon of hope for patients whose therapeutic options have been exhausted but also as a valued resource accessible by and contributing to the nation's clinical research enterprise.

What's Required?

To succeed in meeting the vision for a strong Clinical Center in the future that continues to make new discoveries about human disease leading to new therapies, we need active leadership of a strategic plan that encompasses NIH intramural clinical research programs. This form of coordination and priority-setting, which in my opinion is long overdue, will result in recruitment of more and better tenure-track clinical investigators and bring new extramural partners to our community while contributing to the needs of colleagues in academia and industry.

—John Gallin
Director, NIH Clinical Center

---

1. N.S. Sung, W.F. Crowley, M. Genel, M., et al. "Central challenges facing the national clinical research enterprise," *JAMA*. **289**, 1278 (2003).

2. E. Zerhouni, "Translational and clinical science—time for a new vision," *NEJM* ; **353**, 1621 (2005)

3. J. Rivera and M. Gottesman, "Trans-NIH intramural scientific initiatives," *The NIH Catalyst*, **14** (5), 2 (2006).

4. M. Gottesman, "Turning medical students into physician-scientists," *The NIH Catalyst*, **15** (2), 2 (2007).

ISO: A Bigger Group to Steer NIH SS/SC Organization

The NIH Staff Scientists/Staff Clinicians (SS/SC) Organization is seeking to expand its newly formed Steering Committee.

Currently comprised of at least one representative from each of the 22 NIH institutes and centers (ICs) with intramural research programs, the Steering Committee is now aiming to recruit a total of two representatives for each IC—one staff scientist and one staff clinician—to ensure that the needs of both positions are addressed. Each representative will serve as the point of contact for their institute colleagues and as the liaison between their institute and the Steering Committee.

The idea of creating an NIH SS/SC organization to address the interests of the more than 1,000 staff scientists and staff clinicians dispersed across NIH arose in 2004. SS/SC employed through the GS-scale, Commissioned Corps, or Title 42, or as contractors are included.

Early discussions centered on the general structure of this pan-NIH organization, as some ICs already had their own SS/SC organization, and identifying areas of common concern that could be addressed through the NIH leadership. Toward that end, Joan Schwartz, assistant OIR director, continues to provide support and guidance.

The Steering Committee seeks to foster the development within each IC of its own Institute SS/SC Organization. This would facilitate better dialogue between staff scientists and staff clinicians and their respective leadership and administration.

As such, the NIH SS/SC Organization would serve as an umbrella organization that

- Represents all NIH staff scientists and staff clinicians
- Provides a forum for the discussion of policies and practices across NIH
- Offers working models of how things are done at different ICs to facilitate implementation in ICs that wish to adopt similar strategies
- Organizes seminars and workshops on cutting-edge or emerging technologies
- Fosters career development through seminars and workshops on such topics as management, mentoring, and lab organization
- Promotes networking and interinstitute collaborations through the creation of a database of resources and areas of expertise of individual SS/SC organizations.

For more information, visit the webpage: **<http://tango01.cit.nih.gov/sig/home.taf?_function=main&SIGInfo_SIGID=145>**, including the names of IC representatives serving on the Steering Committee, as well as agendas and minutes of past meetings.

Individuals are strongly encouraged to contact their IC representative to volunteer to serve as a representative, to help organize their IC SS/SC Organization, or to suggest concerns that should be raised before the committee.

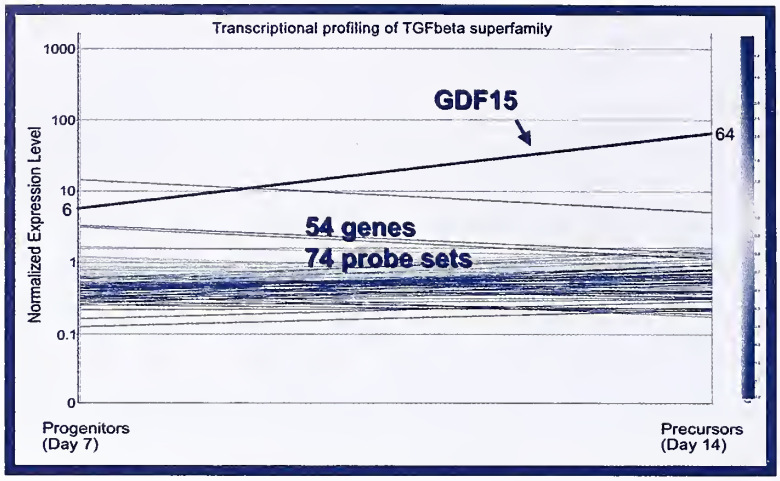
—Michael Difilippantonio & Ofelia Olivero

Iron Regulation

continued from page 1

a very unusual mechanism in the erythroid cells, what we may have discovered is a very generalizable mechanism of iron regulation, or of how tissue damage can activate certain hormones, whether it be from cancer or from liver disease or, in our case, from anemia," Miller said. "One discovery in a patient with thalassemia is now leading to other discoveries in other patients."

The discovery capitalized on the unique blend of resources at NIH, such as expertise in bioinformatics, the NIH Intramural Sequencing Center (NISC), collaborations with smart lab neighbors, and patients at the Clinical Center.



from Tanno, et al. (2007)

***Identification of GDF15 as the candidate molecule**: After transcriptional profiling of the TGF-β superfamily members in erythroblasts, GDF15 stood out among 54 genes. GDF15 suppresses the iron regulator hepcidin, and later it was revealed that patients with thalassemia demonstrated 10-100–fold increases in serum GDF15 levels when compared with healthy volunteers*

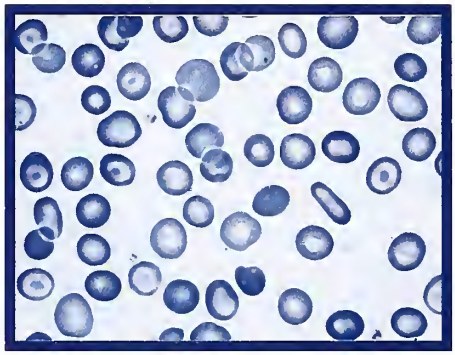
From Dinner To Bench to Bedside

Miller, a hematologist, studies a variety of blood diseases. The core of his lab's research since the mid-1990s has been to map gene expression in the erythroid lineage.

"We have spent a decade trying to put together an infrastructure for all of the genes that are being turned on and off as an adult stem cell becomes a red [blood] cell," Miller said. "This information database allowed us to correlate clinical observations with animal models and allowed us to ask what signals could possibly regulate iron."

What hooked Miller on iron was a conversation about a year ago at a dinner with the local chapter of the Cooley's Anemia Foundation, an advocacy group with members suffering from this form of thalassemia.

Cooley's anemia is an autosomal-recessive blood disease affecting only about a thousand people nationally, although single-gene carriers for thalassemia are found in 10 percent or more of the population in Thailand, Cyprus, and other malarial regions. People with thalassemia are unable to properly synthesize one of the globin chains that make up hemoglobin and are thus prone to anemia. They have elevated numbers of erythroblasts in their bone marrow but reduced numbers of healthy erythrocytes circulating in the blood. Cooley's anemia and other forms of thalassemia are treated primarily by frequent blood transfusions.



Barbara Bryant

***Bull's eye:** Peripheral blood smear from a donor with thalassemia trait captures classic telltale "target cells"—red cells that have a central bull's eye*

The advocacy group told Miller its greatest concern was iron overload, a main underlying cause of death for those with thalassemia. The excess iron can stunt growth, destroy the thyroid gland, damage the liver, and induce diabetes, to name a few complications. Doctors have known that, even in the absence of transfusions, thalassemia patients often accumulate iron—and transfusions markedly worsen this.

"Maybe there is something about the way they make their blood—incorrectly—that makes them absorb too much iron," Miller hypothesized.

Miller had hints from several sources—including the clinic he established at the Clinical Center and an animal model from the neighboring lab of Chuxia Deng, chief of the NIDDK Mammalian Genetics Section—that the signal could be a member of the transforming growth factor–β (TGF-β) superfamily of genes. He was particularly inspired by Deng's work, which he had heard at a NIDDK retreat, and began to concentrate on TGF-β proteins, including GDF and bone morphogenetic proteins, all in the superfamily. He was also inspired to pursue the project by Carolyn Philpott, senior investigator in NIDDK's Liver Diseases Branch and an expert on iron biology.

Alan Schechter, the NIDDK Molecular Medicine Branch chief, described Miller's lab's process of narrowing in on the suspected genes as going from "bedside to bench to bedside," one of the true advantages of the intramural research program.

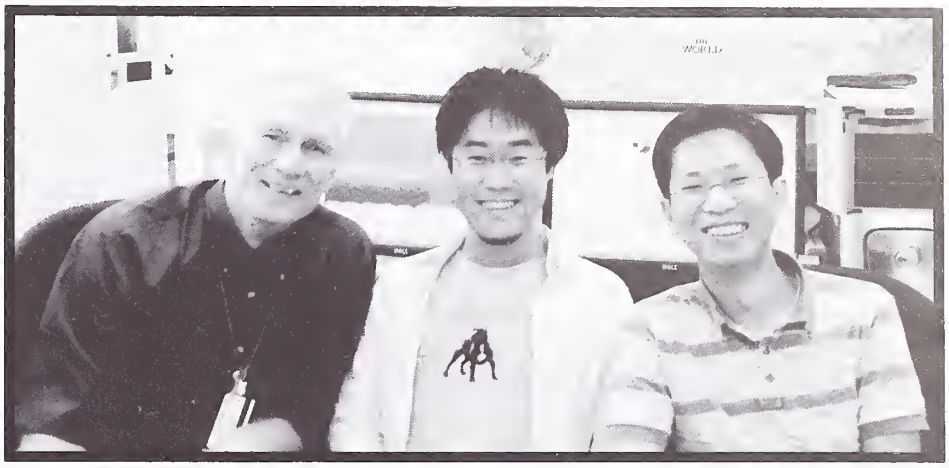
"Jeff established two years ago a clinic in the hospital to see patients," Schechter said. "Once you have the robust clinical base, then you can go back and forth. . . . It's an iterative process. You can't just think some profound thought in a laboratory and then come over to the hospital in the middle of the night to apply it to patients."

Erythroid Gene Clearinghouse

Tanno, an IRTA postdoc fellow, began the project with the meticulous process of identifying all the signals made by adult stem cells during their transformation from erythroblasts to red blood cells. Key to this work was the warehouse of data from the Human Genome Project and bioinformatics tools to analyze these data. A variety of bioinformatic approaches was used, including those available through NCBI, commercial software for array analyses, and custom programming strategies crafted by Gerard Bouffard of NHGRI, director of the NISC Bioinformatics Group.

The process entailed isolating mRNA from erythroid precursor cells, reverse-transcribing this to cDNA, creating thousands of expressed sequence tags (ESTs), removing contaminants, and then "BLASTing" them using NCBI's BLAST tools to compare them with other gene sequences.

"BLAST is one of those tools you can't imagine modern life without," said Bouffard, who wrote a complementary software program for Miller's group "to distill out gold nuggets" from the BLAST



Christopher Wanjek

***Iron-clad team**: (left to right) lab chief Jeff Miller, senior author; postdoc Toshihiko Tanno, lead author; and lab manager Terry Lee, co-author, seated in front of computer display that summarizes more than one million data points describing gene activity during human erythropoiesis*

results. He has worked with Miller on this project for several years.

All this information is posted at NIDDK's Hembase, **<http://hembase.niddk.nih.gov>**, a public repository of information on erythroid ESTs and also sequences encoding several hundred additional genes with known expression in erythroid cells, organized and linked according to the location of these sequences within the human genome.

From Hint to Confirmation

Guiding Tanno on his needle-in-a-haystack search for an iron regulator in the midst of all these sequences was the clinic- and lab-driven hypothesis that iron regulation involved the TGF-β superfamily. He examined adult human erythroid cells from clinic patients to see whether any TGF-β genes were expressed.

Susan Leitman

Three candidates emerged from a long list, with GDF15 in particular copiously expressed by erythroid cells.

Going back to the clinic, the team checked whether patients with thalassemia had too much GDF15 in their blood. The answer was as clear as a bell.

Alan Schechter

"They had incredibly high levels," Miller said, "a logarithmic increase in the level of this in their blood" relative to the patient's iron overload and compared with healthy patients. "We were thinking, wow. . . . It was very satisfying to be able to go back to the clinic to these patients who were donating their blood to figure out if our hints in the laboratory were useful."

Gerard Bouffard

Once the group found what was increased in the blood, they could determine the mechanism: GDF15 appears to be an inhibitor of hepcidin, a major iron regulator made in the liver. This hepcidin suppression leads to more iron absorption.

"Since erythroblasts need iron to make hemoglobin, we reasoned that the increased number of erythroblasts in thalassemia may send stronger messages to the liver to suppress hepcidin and thereby absorb more iron even in the condition of iron overload," said Tanno.

Broader Implications

Miller has about a dozen thalassemia patients in his clinics, but he has been able to strengthen his ongoing research on this topic by studying blood from other patients, including those with other iron-overload diseases seen by Susan Leitman, chief of the Blood Services Section of the CC's Department of Transfusion Medicine, as well as by receiving samples from thalassemia patients in Thailand, where nearly 1 percent of the population has some thalassemia disease.

"Many experts in iron regulation have postulated the existence of an 'erythroid regulator' of iron absorption for a while now,"said Leitman. "The search for this erythroid regulator has been a hot issue in labs focused on iron homeostasis. Jeff's identification of GDF15 is a major breakthrough in this field."

The Thai samples came courtesy of Suthat Fucharoen of Mahidol University in Nakornpathom, Thailand, who had spent a summer at NIH several years ago and who has continued to collaborate with Miller, Schechter, and NIDDK Director Griffin Rodgers.

"This is the basis of how science works—exchanging visits and established collaborations," Schechter said, adding that Fucharoen's collaboration has greatly aided in the discovery of iron regulation.

There are other anemias that don't behave like thalassemia but still might be regulated by hepcidin and GDF15, Miller said. He also speculates that the involvement of GDF15 in thalassemia and other diseases, including cancers, may extend beyond the regulation of hepcidin.

"We're hoping now—very much so—we can use this for diagnostic, prognostic, and even therapeutic purposes, because the discovery of signals immediately leads to the possibility of blocking those signals," Miller said.

"Information that has come from the study of hemoglobin diseases has been applicable to lots of other diseases," said Schechter, citing Linus Pauling's 1949 paper defining sickle cell as the first known molecular disease. What Miller has done, Schechter added, was make "use of the new era of genomic medicine to define a subfield that probably could be called human erythroid cell genomics . . . to pioneer a whole new field within hematology."

Whereas the current project has been focused almost entirely on thalassemia, the lab hopes to apply this translational approach to other diseases and to ensure that the broader scientific community has access to their genomic studies to facilitate basic and clinical connections that would otherwise be difficult to make.

"All this information can now be organized because of the Human Genome Project. Let's not forget that," Miller said. "Now we're taking the next step—and perhaps the more difficult though most important step—to use the information to make people feel better." ■

---

1. T. Tanno, N.V. Bhanu, P.A. Oneal, S.-H. Goh, P. Staker, Y.T. Lee, et al., "High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin," *Nature Medicine*, published online, August 26, 2007.

NISC: the Sequencing of Events
continued from page 1

with access to large-scale DNA sequencing and sequence analysis.

Start-up funds from the intramural programs of 14 NIH institutes and centers enabled NISC to renovate space and procure equipment for performing large sequencing projects (then defined as a modest 500 DNA sequence reads) on a fee-for-service basis. All 14 have availed themselves of NISC services over the years.

For example, notes NIDCD Director James Battey, NISC staff was instrumental in helping intramural scientists in the NIDCD Laboratory of Molecular Genetics identify several genes whose mutations result in hereditary hearing impairment, as well as two of the first mammalian taste-receptor subunits.

But the center has also evolved beyond its original vision, conducting larger-scale projects that, in turn, enabled its expansion. NISC's current capacity is approximately 6.6 million sequence reads per year, and growing.

Starting in temporary space provided by NIDCD at 5 Research Court in Rockville, NISC has since moved twice as it underwent major growth spurts. It now resides on the top floor of the NIH-rented building at 5625 Fishers Lane in Rockville.

A spacious and bustling facility, with a laboratory and computational staff of between 30 and 40 people, NISC produces DNA sequence data 24 hours a day, seven days a week.

The sequencing center also is frequently toured by government and classroom contingents in search of a connection between advancing technology and biological research.

"NISC provides a valuable focal point for genomic education and outreach at NIH," observes Eric Green, NHGRI scientific director and NISC director. "We regard such outreach as part of NISC's mission."

NISC, says Green, "packs a lot of power in a midsized punch."

In addition to working with NIH investigators, NISC collaborates with other leading genomics programs in this country and abroad, including the sequencing centers at the Eli and Edythe L. Broad Institute of the Massachusetts Institute of Technology and Harvard University in Cambridge, Mass.; Baylor College of Medicine in Houston; Washington University in St. Louis, Mo.; and the Wellcome Trust Sanger Institute in Cambridge, UK.

Enter Green

Since Green arrived at NHGRI from Washington University in 1994, his research program has focused on mapping, sequencing, and interpreting vertebrate genomes.

He brought one of the first comparative sequencing projects to NISC—sequencing and studying the region encompassing the cystic fibrosis gene in the mouse genome. The center's participation in three subsequent projects—sequencing the mouse genome, the Cancer Genome Anatomy Project, and the Mammalian Gene Collection Program—catalyzed a major NISC expansion.

"The net effect," Green said, "was the establishment of an efficient genome-

Maggie Bartlett

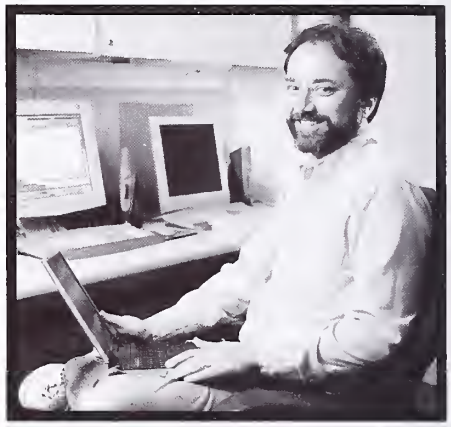
Eric Green

sequencing program that produced extremely high-quality sequence data. Much of NISC's success can be attributed to a staff who routinely implements appropriate approaches and methodologies, in all cases tailored to important scientific projects."

Enter Bouffard

After arriving at NHGRI as a postdoctoral fellow in 1994, Gerard Bouffard, director of the NISC Bioinformatics Group and associate investigator in the NHGRI Genome Technology Branch, witnessed the materialization of Green's concept of a high-throughput DNA sequencing facility at NIH.

"There were DNA-sequencing instruments here and there at different institutes, some of which were heavily overused while others didn't see much use," Bouffard recalled. "Rather than create a traditional sequencing core where someone could drop off any small-scale project, the NISC concept proposed to focus on projects that required the analysis of large numbers of samples."



Maggie Bartlett

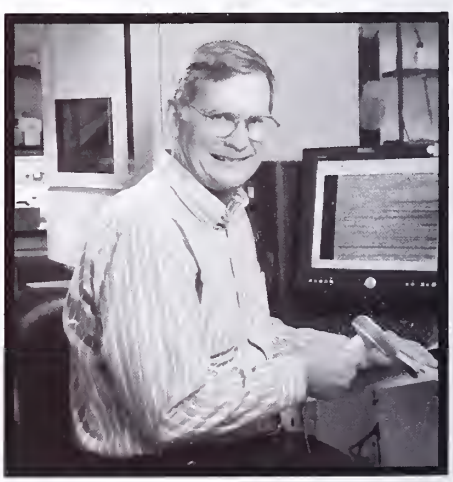
Gerard Bouffard

Under Bouffard's leadership, the NISC Bioinformatics Group grew from an initial staff of two in 1997 to its current 11 members. Bioinformatics relies on computing capabilities, and NISC started with two disc arrays and combined disc space of 200 gigabytes. "In 1997, we thought that was a fantastic amount of disc storage," Bouffard said. "Nowadays you could almost put that in your pocket."

Bouffard recalls that in the early days of NISC, it would take a technician almost as much time to type in the sample names by hand as it did to pour a gel used for sequencing. His group established a naming scheme and a two-letter code system that they thought would last a long time. "As careful as we were to establish this system, the volume of sequencing projects tackled by NISC far exceeded our initial imagination," Bouffard said, referring to the present four-letter code system.

Enter Blakesley

All NISC data are generated by the Sequencing Group, headed by Robert Blakesley, who is also an associate in-



Maggie Bartlett

Robert Blakesley

vestigator in the NHGRI Genomic Technology Branch. Blakesley joined NISC in 2000 after a 20-year career in the biotechnology industry. Some years before, he performed manual DNA sequencing in which gels were put against X-ray film, exposed for a day, and then developed to expose dark bands on the film.

"The great advance at NISC," Blakelsey said, "was that fluorescence was detected by an instrument in an automated fashion using software that converted the primary data to sequence. This eliminated the need for radioactivity and manual interpretation of bands on X-ray film."

Enter Gupta

The most senior technician in the Sequencing Group, Jyoti Gupta, arrived at NISC in 1998, immediately after her senior year as an undergraduate student studying biology. On the last day of classes in 1998, she received e-mail offering her a postbaccalaureate Intramural Research Training Award (IRTA) at NISC. Gupta was then one of just three members of the NISC Sequencing Group; now she manages a team of seven technicians.

Eric Green

Jyoti Gupta

Gupta reflected with some amusement on the early days at NISC. The facility then consisted of three rooms, including a converted storage closet that housed six Applied Biosystems model 377 DNA-sequencing machines. These instruments were gel-based, requiring significant time and care to prepare the gel and to manually track the samples on a computer following the analysis. It was easier to track the sequence data on the computer monitor in the dark, so she and her colleagues used a low-tech cardboard box placed over their heads to help track the samples.

In 2000, capillary-based DNA sequencing systems came on the scene, eliminating the need for gels and the burden of tracking samples on the computer. With these new instruments came a major growth in personnel.

Together, these changes led to a massive increase in the overall sequencing capacity at NISC, allowing the center to become part of the consortium of large-scale sequencing centers and to adopt a focus on comparative sequencing.

The Past is Prologue

"NISC's leadership could see the future growth of large-scale sequencing, especially for generating data that would help us interpret the human genome sequence," Blakesley said. "We staked out a claim that NISC was going to be a comparative sequencing shop."

In fact, according to Blakesley, the initial sequences from various species generated by NISC provided helpful preliminary data that facilitated decisions regarding which other species' genomes to sequence.

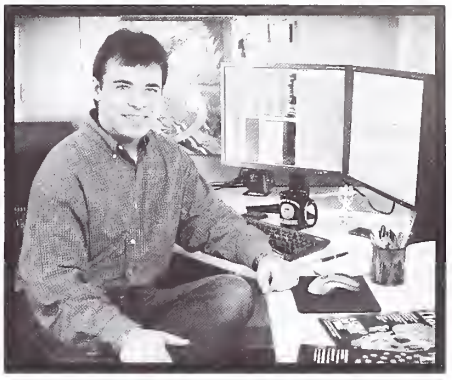
Through the experience gained in sequencing DNA from more than 60 vertebrate species, NISC has established particular expertise in sequence finishing—a specialized activity that requires refinement and perfection of the sequence data.

Sequence finishing can be expensive, but it is essential because it ensures accurate sequence analysis. Numerous problems are routinely encountered during sequence finishing, including establishing whether detected sequence changes are due to evolution or reflect errors in the generated data. This keeps Gupta's group quite busy.

New software tools being developed by NHGRI tenure-track investigator Elliott Margulies, among others, are being used to extract information from the comparative sequence data generated at NISC.

Such analytical methods are integral to projects like ENCODE, which generated trenchant insights from the intense scrutiny of only 1 percent of the human genome. In fact, it was NISC's early forays into comparative sequencing that demonstrated the value of zeroing in on a limited portion of the human genome en route to establishing how to interpret the human genome sequence more broadly.

Over the past 10 years, NISC has cultivated a network of more than 100 national and international scientific collaborators. These collaborations have engaged NISC scientists in a broad array of research projects, ranging from human disease studies to the examination of chromosome structure and evolution. These efforts have led to the publication of more than 60 papers that include NISC staff members as co-authors.



Maggie Bartlett

Elliott Margulies

Currently, in collaboration with investigators around the world, NISC is expanding its capabilities in the area of medical sequencing—that is, sequencing the DNA of patients as part of clinical research projects.

At the Bethesda campus, NISC is partnering with investigators across NIH in a recently launched clinical research project called ClinSeq, which will use cutting-edge high-throughput sequencing to identify genetic variants associated with clinical phenotypes, with an initial focus on cardiovascular disease. ClinSeq aims to deploy large-scale sequencing in a clinical research setting and will allow NISC to become increasingly involved in clinical research efforts at NIH. ■

Celebrating the 10th And Looking to the Future

To commemorate NISC's 10th anniversary, NHGRI—NISC's scientific and administrative home—is holding an all-day symposium, *Genome Exploration by Large-Scale DNA Sequencing: Circa 2007 and Beyond*, on **Oct. 16, 2007**, in Masur Auditorium in the NIH Clinical Center. For details, see **<www.genome.gov/NISC10th>**.

Sequencing Services

NIH researchers may visit the NISC website **<http://www.nisc.nih.gov>** and select "information for investigators" regarding sequencing services (the minimum project size requirement is 768 sequence reads). For more info, Gerry Bouffard may also be contacted at **<bouffard@mail.nih.gov>**.

Summer Poster Day: Displaying the Rewards of Student-Mentor Teamwork

Summer Research Poster day, held this year August 1, showcased 725 posters. Following is some of the work from two labs, the NCCAM Laboratory of Clinical Investigation and the CC Scientific Computing Section.

Plumbing Prostate Cancer Cells

"Effects of siRNA Knockdown of 3β or 17β Hydroxy-Steroid Dehydrogenases on PSA Production in Prostate Stromal and Epithelial Cell Cocultures Treated with DHEA and TGFβ," **Sweta Sharma**, The George Washington University, Washington, D.C., Xunxian Liu, and Julia Arnold, Laboratory of Clinical Investigation, Endocrine Section, NCCAM.

"Nature vs. Nurture?: How PSA Production in Cancer Epithelial Cells from the Human Prostate Gland may be Regulated by Factors Released by Its Supporting Stromal Environment," **Patricia Reutemann**, GWU Medical School, Nora E. Gray, Xunxian Liu, Marc R. Blackman, and Julia T. Arnold, Endocrine Section, NCCAM

Christopher Wanjek

***NCCAM quartet**: Students Sweta Sharma (second from left) and Patricia Reutemann (second from right), flanked by mentors Xunxian Liu (left) and Julia Arnold*

Patty Reutemann and Sweta Sharma of George Washington University (GWU) explored possible mechanisms of how prostate stromal cells can affect prostate epithelial cancer cells, particularly in the presence of dehydroepiandrosterone (DHEA).

DHEA is an endogenous hormone made by the adrenal gland in high concentrations, although levels decrease with age. DHEA is thus used commonly as a dietary supplement for purported anti-aging benefits.

In the prostate, DHEA can transform into androgens or estrogens, and this transformation may affect prostate pathophysiology.

NCCAM's Laboratory of Clinical Investigation has tested the effects of DHEA on various human prostate cell models and has found unique mechanisms of DHEA when two different cell types are cultured together.

DHEA's effect on prostate cancer cells is minimal when they are grown alone, but when the prostate stromal cells—the cells from associated prostate connective tissue—are included, DHEA's effects are accentuated.

The addition of TGFβ-1 induces reactive prostate stroma, similar to stroma associated with inflammation in the cancer tissue microenvironment.

Earlier NCCAM research found increased metabolism of DHEA towards androgens when TGFβ-1 is added, as measured by increased prostate-specific antigen (PSA) production in the cancer cells.

Reutemann, who is entering her second year at GWU medical school with a concentration in integrative medicine, used a real-time PCR array targeting 84 human growth factor genes to search for potential secondary paracrine factors expressed by the stromal cells that may contribute to the androgenic effect on the epithelial cells. Included in her results were andromedins, such as IGF-1, FGF-1, and FGF-7.

Sharma, a GWU undergraduate studying biology and computer science, targeted the stromal metabolism of DHEA to androgens or estrogens by using a silencing RNA approach for the enzymes involved in steroid metabolism.

She showed that silencing either 17β hydroxysteroid dehydrogenase type 1 or type 5 in the stromal cells reduced the epithelial cell PSA production in these co-cultures. These enzymes promote the conversion of DHEA into testosterone, which may in turn fuel the prostate cancer's growth.

Reutemann said her CAM-infused research at medical school "is the best of both worlds," providing disciplined research training in the field of botanical and traditional medicines, a field she has long admired. The internship meshed well with her university's requirements.

Sharma is unsure about her academic path, but she noted that the internship introduced her to the trouble-shooting, frustration, and, ultimately, joy that is bench work.

—Christopher Wanjek

October Meeting: Cancer & Inflammation

A meeting on "Cancer & Inflammation," sponsored by the NCI Center of Excellence in Immunology, will take place **October 9–10** at the Masur Auditorium, Building 10.

Session topics include innate resistance and cancer, colon and prostate cancers, skin cancers, cancers with an infectious pathogenesis, and the inflammatory tumor microenvironment.

Registration is free, but seating is limited. For more information, contact Karen Kochersberger at 301-228-4027 or **<kochersberger@ncifcrf.gov>**.

Register online at **<http://web.ncifcrf.gov/events/cancerandinflammation/>**.

Computation in Clinical Research

"The Importance of Prevalence and Misclassification Cost Considerations in Computerized Clinical Decision Support Systems," **Ina Sen**, Arizona State University, Tempe, James DeLeo, Scientific Computing Section, Department of Clinical Research Informatics, CC

Ina Sen worked at the Clinical Center this summer developing a suite of software applications to help clinical researchers better visualize and analyze patient data.

She worked with three other summer interns under the direction of James DeLeo, chief of the Scientific Computing Section in the CC's Department of Clinical Research Informatics.

Her team's project—which came through DeLeo as a request from Fred Miller, a senior investigator with NIEHS at the CC—was to develop a computer system to merge disparate yet related datasets and to display graphically the combined information, such as analyte values from numerous patients.

The goal is to provide a desktop tool that enables researchers to create snapshots of data as parallel coordinates or star glyphs, to chart changes over time, mine data, perform statistical analyses, and potentially reveal unrealized connections among data.

Sen is a graduate of the Indian Institute of Technology Roorkee, Uttaranchal, and is now a third-year doctoral candidate at Arizona State University studying computer science with a concentration in bioinformatics and machine learning.

One of her tasks on the team was to participate in the design of computational classifiers for both supervised and unsupervised computer learning. Supervised learning would entail using factors such as analyte values associated with confirmed diagnosed cases to teach the computer to classify new cases as normal or diseased.

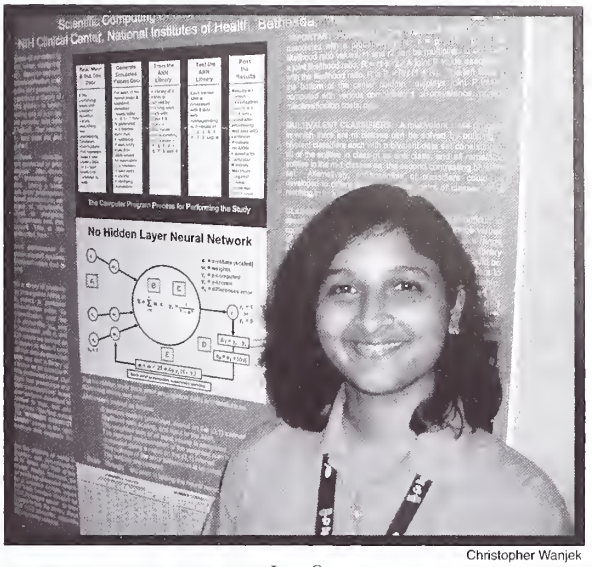
In unsupervised learning, cases are unlabeled—that is, diagnostic categories are either unknown or assumed to be unknown, and the computer uses feature values to cluster cases, resulting in confirmation of existing diagnostic categories or suggestions of new ones.

Sen said that computational classifier methodologies often do not consider prevalence—that is, an expected or prior probability of events—or they ignore the potentially dire consequence of misclassifying an event in candidate classes. Thus, one focus of her work was to demonstrate the importance of considering prevalence and misclassification costs.

Her analysis, based on the output of real CC data processed through an artificial neural network, indeed supported this hypothesis, and Sen recommends that consideration of these two factors be incorporated in computational classifiers.

DeLeo and his colleague Carl Leonard in the Scientific Computing Section will continue this software development with Miller and look for opportunities to leverage the experience gained on this project to support other clinical investigators in a similar manner.

—Christopher Wanjek



Christopher Wanjek

Ina Sen

***Keeping it together**: Carl Leonard (left) and Jim DeLeo (right) stand with the scientific computing summer-student team, each of whom presented a poster on Poster Day—Alexander Senf (top row, left), doctoral candidate in computer science at the University of Kansas, Lawrence ("Statistical Knowledge Discovery in Medical Data Sources"); James Stoner (top right), undergraduate at the University of Maryland, Baltimore County ("Gathering, Formatting, and Using Biomedical Data from Disparate Sources to Reveal Medical Knowledge"); Ina Sen, and Maria Balarezo, student at Gaithersburg High School in Gaithersburg, Md., ("Identification and Analysis of Idiopathic Inflammatory Myopathies")*

Interest Group Updates

The **Neurobiology Interest Group** has new contact people. Its IG directory listing is now

Neurobiology Interest Group
Meeting time and place: Check website
Contact 1: Jeff Diamond
Phone: 301-435-1896
E-mail: <DiamondJ@ninds.nih.gov>
Contact 2: Mark Stopfer
Phone: 301-451-4534
E-mail: <stopferm@mail.nih.gov>

The **Mucosal Immunology Interest Group** will hold its first meeting Friday, **October 19**, from 12:00 to 1:00 pm in the first-floor conference room (room 1201) of Building 40 (the VRC).

Thereafter, the regular meeting day for the MIIG will be the last Friday of the month. The updated listing follows:

Mucosal Immunology Interest Group
Meeting time: Monthly, last Friday
Meeting place: Building 40, room 1201 (First-Floor Conference Room, VRC)
Contact 1: Brian Kelsall
Phone: 301-496-7473
E-mail: <bkelsall@mail.nih.gov>
Contact 2: Yasmine Belkaid
Phone: 301-451-8686
E-mail: <ybelkaid@mail.nih.gov>
Contact 3: Warren Strober
Phone: 301-496-6810
E-mail: <wstrober@mail.nih.gov>

The Shining Legacy of Anita Roberts

by Gail Seabold and Holly Dimitropoulos

The Anita B. Roberts Lecture Series was established by the Women Scientist Advisors (WSA) Committee in 2006. The series is dedicated to the memory of Anita Roberts, chief of the Laboratory of Cell Regulation and Carcinogenesis at NCI from 1995 to 2006.

Roberts spent 30 years at NIH, arriving at NCI in 1976 after completing her postdoctoral fellowship at Harvard University. She obtained her Ph.D. in biochemistry from the University of Wisconsin in 1968.

In March 2004, she was diagnosed with aggressive stage IV gastric cancer, which she fought for two years, until her death on May 26, 2006. She documented her thoughts, feelings, and experiences—as a scientist, a family member, and a cancer patient—throughout those two-plus years in her blog:
<http://www.anitaroberts.net>

Roberts as Scientist and Mentor

Anita Roberts was recognized for her combination of excellence as an investigator, mentor, and leader in the scientific community. She received many awards and acknowledgements from her peers, such as the Susan G. Komen Foundation Brinker Award for Distinguished Science, the FASEB Excellence in Science Award, and the Leopold Griffuel Prize.

She was a pioneer in characterizing and showing the significance of transforming growth factor–β (TGF-β) in critical cellular processes such as wound healing and the regulation of cancer, particularly the enhancement of metastasis.

This molecule, to which Roberts devoted her professional life, was later cloned in collaboration with Genentech and expressed in bacteria. The pathway that TGF-β regulates is now considered a target for cancer and rheumatoid arthritis therapies, among others.

From 1983 to 2002, Roberts was one of only three female scientists among the 50 most-cited researchers in the world, compiled by Thomson Scientific's *Science Watch* in a feature called "Twenty Years of Citation Superstars."

This honor and achievement speaks for itself in terms of the quality of work she and her laboratory staff produced. Her lab's productivity was also a testament to the nurturing environment she provided.

NCI's Michael Sporn, who hired Roberts in 1976 to collaborate with him on vitamin A research, also cited the accomplishments of this brilliant scientist and devoted wife, mother, and, eventually, grandmother as shining testimony that "it is possible for a woman to have a very successful, competitive, cutting-edge career and also have an excellent family life."

Honorees Thus Far

The goal of the Anita B. Roberts Lecture Series is to highlight the research achievements of women scientists in the NIH intramural research program. The first two speakers were Elizabeth Nabel and Elaine Jaffe.

Nabel, NHLBI director and chief of the Vascular Biology and Genomics Section, NHGRI, spoke on "Genomic Medicine and Cardiovascular Disease." Her research focuses on defining pathways that regulate cell growth in the vasculature, remodel the vasculature after injury, and lead to genetic susceptibility to vascular diseases.

Jaffe, chief of the Hematopathology Section and acting chief of the Laboratory of Pathology, NCI, discussed "The Many Guises and Disguises of Follicular Lymphoma." Jaffe is an internationally recognized expert in the classification and differential diagnosis of lymphoma; her work has highlighted the role of the clinical pathologist in the accurate management of these myriad diseases.

The next speaker will be NIDA Director Nora Volkow, whose research shows that blood flow, dopamine levels, and glucose metabolism all change in the brains of chronic drug users—but can return to their predrug state with abstinence.

She explains in the July 16, 2007, issue of *Time* magazine that "some people have a genetic predisposition to addiction, but because it involves these basic brain functions, everyone will become an addict if sufficiently exposed to drugs or alcohol." Volkow further states that "addiction is a medical condition. We have to recognize that medications can reverse the pathology of the disease. We have to force ourselves to think about a cure [for addiction] because if we don't, it will never happen."

Anita Roberts

Please join us on **October 23, 2007**, at 1:30 p.m, in the Lipsett Amphitheater, Building 10, for Volkow's lecture, "Why Is It so Hard for the Addict's Brain to Just Say No?" to learn more about addiction and the current progress that has been made in treating it. Following the lecture, there will be a question-and-answer session addressing career issues for women in science. ■

Series Open to the Public

The Anita B. Roberts Lecture Series is sponsored by the NIH Women Scientist Advisors Committee and the Office of Research on Women's Health. The lectures are open to the public, and sign-language interpreters are provided on request.

Individuals with disabilities who need reasonable accommodation to participate should contact Dierdre Andrews, 301-496-3891, or Federal Relay, 1-800-877-8339, five days before the lecture. ■

Training Page: From the Director, Office of Intramural Training and Education

Trainees Looking for Success

We had invited six tenured investigators to an informal panel discussion with our young trainees, most of whom are trying to figure out what to do with their lives (a process, I tell them, that doesn't end at graduation). The event was scheduled for August 14 at Natcher.

This was the first OITE mentoring panel for NIH trainees, followed by lunch and a poster session so that students could meet and talk with our tenure-track investigators, and we'd planned for about 100 trainees. More than 200 students and postbacs poured into the room.

Initially we discussed characteristics of successful trainees. Panelist Polly Matzinger of NIAID set the tone. "We are the biggest biomedical research facility on the planet," she said of the opportunity afforded to the trainees. To succeed, she advised, one must be a "courageous masochist . . . because, at its best, research is 90 percent failure."

Les Biesecker of NHGRI echoed this sentiment, saying that an accomplished researcher will proceed "from failure to failure to failure with unprecedented optimism," a line he attributes to Stephen Emerson, president of Haverford College.

Sage advice (and dry humor) came also from the youngest on the panel, the newly tenured Danny Douek of the VRC. "Know your limitations, and ask when you don't know," he said. "And understand that advice is not criticism." And take the advice, he advised.

Sharon Milgram
OITE director

Several trainees expressed worry that they weren't on target with career goals. Barbara Vonderhaar of NCI told them to remain open to new ideas and new career possibilities. And Ken Fischbeck of NINDS added that there are many paths to a career in research; all the panelists encouraged the students to explore various career options—and, of course, to take advantage of all NIH has to offer.

Questions from the trainees ranged from how to plan a family and how to plan a divorce—from a research project going nowhere. This question brought winces from panel members, as each replayed in his or her own mind some scientific disappointment.

Roland Owens of NIDDK said such a decision is difficult for even the most experienced researcher. Douek described such a situation as being in a bad relationship—you're too close to think rationally. He recommended seeking "outside advice" from colleagues.

The answer to many questions focused on developing a strong network of mentors and on seeking advice from many investigators.

By the end of the discussion, the panel was quoting Confucius and speaking of science as an art form.

After the hour discussion we proceeded to a box lunch and tenure-track investigator poster session. Yosuke Mukoyama of NHLBI, one of the poster presenters, called the event an excellent opportunity to meet and recruit young talent. So information clearly flowed both ways.

Please keep tuned for future trainee events by visiting our website—**<http://www.training.nih.gov>**. Importantly, encourage your trainees to avail themselves of your institute's training office and OITE's services. ■

From the Fellows Committee

Job Fair Calling

The NIH Research Festival, now in its 20th year, will be held **September 25–28**. Aside from the great opportunity to see some of the best in NIH intramural science, the festival includes the Job Fair for NIH Postdoctoral, Research, and Clinical Fellows on Thursday, September 27, from 11:30 a.m. to 3:00 p.m. at Natcher.

The NIH Fellows Committee (FELCOM) is co-sponsoring the job fair with the NIH Office of Intramural Training and Education (OITE) and the Office of Research on Women's Health (ORWH).

Last year's job fair was a great success, with more than 40 organizations represented and nearly 1,000 fellows attending. We expect a similar turnout this year.

This year's is the biggest job fair ever at NIH, and we highly recommend that NIH fellows attend—regardless of what stage you are in your training—just to make connections, familiarize yourself with the job market, and take the next step in your career. If you do attend, remember to dress professionally and to bring several copies of your résumé.

NIH Director Elias Zerhouni will give the plenary address at the job fair, entitled "The Future Direction of Biomedical Research," from 10:30 a.m. to 11:30 a.m. at Natcher. A continental breakfast and box lunches will be available on a first-come-first-served basis. More information about the job fair is at **<http://www.training.nih.gov/jobfair>**, including job postings at **<http://www.training.nih.gov/onlineapps/jobFair/application/participants.asp>**.

FELCOM is also hosting the FARE 2008 award ceremony and reception on **September 24** at 4:15 p.m. at Natcher.

FARE is an annual NIH-wide competition in which winning fellows receive a $1,000 travel grant. Information about this excellent funding opportunity is at **<http://felcom.nih.gov/FARE>**.

FELCOM represents the collective body of NIH postdoctoral and clinical fellows. The committee comprises fellows in both clinical and basic science from each of the institutes and centers as well as from FDA-CBER and USUHS.

The committee serves as a liaison between fellows and NIH administrative bodies and has ad hoc representation on several bodies, such as the NIH Training Directors Committee and the Graduate Medical Education Committee.

We meet on the first Thursday of every month at 4:00 p.m. in Building 1, usually in Room 151. Anyone at NIH can attend.

This *Catalyst* column will serve as a recurring forum to highlight FELCOM activities. Our website is **<http://felcom.nih.gov>**.

—Lori Bibb
FELCOM Publicity Subcommittee

On Tenure Track

Christopher Wanjek

Cuilin Zhang

Cuilin Zhang is an epidemiologist focusing on genetic and lifestyle risk factors influencing gestational diabetes and chronic metabolic diseases. She joined the NICHD Epidemiology Branch in June.

Zhang is a graduate of Beijing Medical University, and she earned her M.P.H. and a Ph.D. in epidemiology from the University of Washington in Seattle. Continuing to work her way eastward, she was a researcher at Harvard School of Public Health for three years before coming to NIH.

Zhang says that recent developments in high-throughput biotechnology and genomic science have allowed—and challenged—epidemiologists to go beyond the assessment of environmental risk factors to sensibly incorporate, analyze, and interpret emerging genetic data in the context of other epidemiologic variables.

She hopes, therefore, to partner with geneticists, metabolic biologists, and behavioral scientists at NIH to more fully investigate the interplay of genetic and nongenetic biological markers and lifestyle choices.

Her research activities thus far can be broadly characterized as clinically based case-control and prospective studies on genetic and biochemical markers for gestational diabetes and pre-eclampsia; large-scale prospective studies on lifestyle and gestational diabetes; longitudinal studies on long-term implications of vascular and metabolic disorders in pregnancy; and nested case-control studies of the relationships among genetics, biomarkers, diet, and lifestyle and the risk of type 2 diabetes and related cardiovascular complications.

Zhang calls pregnancy a "stress test" for women predisposed to chronic metabolic diseases. By studying pregnant women she hopes to elicit not only the etiology of metabolic disorders in pregnancy but also underlying biological changes that lead to early stages of type 2 diabetes and cardiovascular disease.

Her research on gestational diabetes has examined the diet and lifestyle of women before and after pregnancy. Based on data from the Nurses' Health Study II, Zhang and her Harvard colleagues found that women who were more physically active and had a prudent diet before pregnancy had significantly lower risk of gestational diabetes. For example, they found that each 10-gm rise in daily fiber consumption was associated with 26 percent reduced risk.

Zhang hopes to continue to understand the proper "dose" of prevention of pregnancy-related complications for women hoping to become pregnant.

—Christopher Wanjek

Christopher Wanjek

Ondine von Ehrenstein

Ondine von Ehrenstein joined the NICHD's Division of Epidemiology, Statistics & Prevention Research this summer, bringing with her many years of expertise in international epidemiology and risk assessment in the area of childhood diseases, with an emphasis on environmental exposure.

Among her many projects is a study examining the relationship between childhood respiratory and atopic illnesses and various environmental and lifestyle factors, including indoor and outdoor air pollution and exposures found in traditional farming environments.

Studying developing and transitional countries, where exposures can be much higher and differ from those in highly developed countries, can provide new insights into disease etiologies and risks, she said.

Von Ehrenstein hopes to study novel biomarkers for exposure, particularly in pregnancy and early childhood, with noninvasive specimen collections. She is also pursuing further analyses of the NICHD Collaborative Perinatal Project, a classic longitudinal study from 1959 to 1974 that followed nearly 60,000 pregnancies and births until the children were seven years old, primarily designed to assess neurodevelopment. This study is a rich source of biomedical, environmental, and socioeconomic information.

Before arriving at NICHD, von Ehrenstein was a researcher for more than four years at the University of California, Berkeley, where she directed research on arsenic exposure and childhood development and reproduction in West Bengal, India.

Her group found a sixfold increase in stillbirth for mothers exposed to arsenic from well water, as well as reductions in cognitive development in school-aged children.

Earlier, during her tenure as a scientist and program manager at the WHO Regional Office for Europe in Rome, she co-edited "Children's Health and Environment: A Review of Evidence."

This 220-page report outlined environmental risks to children who—because of their unique susceptibility, such as a higher skin-to-body-weight ratio and exposures occurring during critical windows of development—suffer from up to 40 percent of the global burden of disease attributable to environmental factors.

Von Ehrenstein conducted her doctoral work in epidemiology at the Children's Hospital of the University of Munich and at the School of Public Health in Bielefeld, Germany, and she has a background in laboratory biology and public health.

—Christopher Wanjek

Kristin Tarbell joined the NIDDK Diabetes Branch in July this year, establishing her own laboratory where she will continue her research in T-cell tolerance and how this goes awry in autoimmune diseases such as type 1 diabetes.

Julie Wallace

Kristin Tarbell

Normally, T cells that recognize antigens from the body are inactivated, thus reducing the response against self and developing specificity against foreign antigens. In autoimmune disease, however, T cells that recognize self are not inactivated; in the case of type 1 diabetes, they proceed to attack the host's own insulin-producing pancreatic β–cells.

While a postdoc at Rockefeller University, Tarbell and her advisor, Ralph Steinman, demonstrated that increases in the number of regulatory T cells could protect insulin-producing β–cells in mice from attack by their own immune system. Regulatory T cells are a rare cell type that can block the response of other T cells and thus could be key to controlling autoimmune diseases. These results suggested that autoimmunity could be reversed and brought to light the role of dendritic cells in this type of T-cell tolerance.

Tarbell's research here will focus on understanding how regulatory T cells work and the role that dendritic cells play in promoting tolerance of T cells. She will explore the signals between regulatory T cells and dendritic cells. In addition, Tarbell will address the development of autoimmunity and how the immune system environment, specifically the role of dendritic cells, differs to allow tolerance of T cells targeting self-antigens.

Drawing on NIDDK's unique and extensive resource of samples from patients with type 1 diabetes, Tarbell also plans to expand her current research to develop better T-cell assays to facilitate the study of the immune system's response against pancreatic cell antigens in type 1 diabetes. These responses, although enough to cause disease, are currently tough to measure, says Tarbell. Improvement in these types of assays will assist in translating Tarbell's results in mice to human patients.

—Julie Wallace

New NIDA Journal Spans Addiction Research and Clinical Practice

Beginning with the November 2007 issue, NIDA's journal *Perspectives* becomes the *Journal of Addiction Science & Clinical Practice*. The peer-reviewed scientific journal—the most widely distributed journal on addiction science—will be published twice a year and will be included in NLM's MEDLINE, broadening its accessibility and reflecting NIDA's commitment, says Director Nora Volkow, "to bringing the latest in addiction science from the laboratory to clinical field as quickly as possible."

Each issue will include:

- Up-to-the-minute reviews by leading researchers of critical topics in the science of drug-abuse prevention and treatment
- Service providers' perspectives on what can and does work in diverse community treatment settings
- Panel discussions on the practical implications of each article for both researchers and service providers
- Examples of successful research–practice collaborations

Acceptance of submitted articles will be based on scientific peer review as well as editorial judgment regarding suitability for publication in a NIDA journal. Past issues, subscription information, and instructions for author submissions can be found online at **<http://www.drugabuse.gov/perspectives>**.

Demystifying Medicine for Ph.D. and Medical Students, Fellows, and Staff, 2008

The popular Demystifying Medicine course will be held every Tuesday from **January 8 to May 6, 2008**, 4:00 to 6:00 p.m. in the Building 50 ground-floor auditorium. All presentations will be videocast and archived.

Register at the Listserv to receive power points and other background info: **<https://list.nih.gov/archives/demystifyingmed.html>**.

For academic credit, register with FAES. The full schedule will appear in the next *Catalyst* and at the course website: **<http://www1.od.nih.gov/oir/DemystifyingMed/index.html>**.

Functional Genomics of Critical Illness and Injury: Meeting Reminder

The fifth symposium on the Functional genomics of Critical Illness and Injury—"Forging a Critical Alliance: Are We Meeting the Need?—will be held **November 14–15, 2007** (8:00 a.m. to 6:30 p.m. and 8:00 a.m. to 5:30 p.m.) at the Natcher Conference Center. The conference is sponsored by NIGMS, the CC Critical Care Medicine Department, and the Critical Illness and Injury Interest Group.

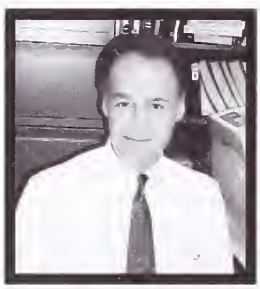
Scientific presentations are scheduled for the first day and collaborative workshops the second day.

Registration closes **October 15**. For additional information, go to: **<http://www.strategicresults.com/fg5>**.

Recently Tenured

Michael R. Bishop *received his M.D. degree from the University of Illinois College of Medicine–Chicago in 1985. He completed an internal medicine residency at Northwestern Memorial Hospital (Chicago) and a fellowship in hematology and oncology at the Loyola University Medical Center in Maywood, Ill. He was an associate professor and director of the Leukemia and Allogeneic Stem Cell Transplantation Programs at the University of Nebraska Medical Center, Omaha, before joining the NCI Experimental Transplantation and Immunology Branch in 1999 as clinical head of the Stem Cell Transplantation Program. He is currently a senior investigator and head of the Transplant Clinical Research Section in that branch.*

My translational research has focused on allogeneic hematopoietic stem-cell transplantation. It has been our goal to increase the understanding of the biology related to the engraftment of allogeneic hematopoietic stem cells.



Fran Pollner

Michael Bishop

Based on murine data, we developed a novel treatment approach, which we refer to as targeted immune depletion, that permits individualized host conditioning according to immune status before transplantation. We were able to demonstrate that targeted immune depletion of host T cells permits the rapid and complete engraftment of allogeneic stem cells. We also demonstrated that circulatory T-cell numbers are a surrogate determinant of graft rejection risk.

As an extension of this work, we recently conducted a prospective clinical trial to determine the effects of targeted immune depletion on IL-7 and IL-15 in the allogeneic transplant setting. As we'd anticipated, we found that IL-7 and IL-15 levels rose from baseline in response to lymphopenia induced by targeted immune depletion and then decreased with lymphocyte recovery post-transplant.

We are now testing the targeted immune depletion approach with HLA-matched unrelated donors in a trans-institutional effort, supported by the National Marrow Donor Program, to extend transplant availability to more patients.

My second area of research has focused on optimizing the graft-versus-tumor (GVT) effect. Based on murine models, we hypothesized that allogeneic cellular therapy would result in a clinically significant GVT effect against metastatic breast cancer.

Our first clinical study was designed to separate the cytotoxic effects of the conditioning regimen from a potential GVT effect by T-cell depleting the allograft and then infusing allogeneic lymphocytes at a later time. This was the first trial to demonstrate a distinct GVT effect against metastatic breast cancer. We are now using Th2/Tc2 cells in our continuing research to enhance the GVT effect against breast cancer.

We are also studying ways to enhance the GVT effect against B-cell malignancies. Relapse is a major cause of treatment failure for patients with non-Hodgkin's lymphomas (NHL) undergoing allogeneic hematopoietic stem-cell transplantation. The standard treatment for relapse after transplant is the infusion of additional allogeneic lymphocytes, referred to as a donor lymphocyte infusion (DLI). However, DLI has limited efficacy against most NHL.

Joe Hinnebusch

Over the past two years, we have engaged in preclinical research to develop tumor-derived lymphocytes to treat recurrent disease after allogeneic hematopoietic stem-cell transplantation. We focused on methods of tumor preparation, based on a platform costimulation with CD3/CD28 beads and expansion with IL-2.

These studies have led to an FDA-approved pilot trial of allogeneic tumor-derived lymphocytes for the treatment of relapsed B-cell malignancies after allogeneic stem-cell transplant.

Our other effort in the enhancement of GVT effects in B-cell malignancies involves the use of idiotype (Id) vaccines in the treatment of multiple myeloma.

In collaboration with the laboratories of Larry Kwak and Sattva Neelapu at the M.D. Anderson Cancer Center in Houston, Texas, we have been conducting a pilot trial on sibling donor vaccination with Id-KLH, derived from the recipient before their allogeneic stem-cell transplant.

The results of this recently completed pilot trial demonstrated that donors could be safely vaccinated and that the large majority of donors had T-cell and/or B-cell responses to Id. Currently we are planning to expand the patient cohort from the pilot trial, and we are also planning to use Id vaccinations as part of transplant protocols for follicular lymphomas.

B. Joseph Hinnebusch *received his Ph.D. in microbiology from the University of Texas Health Science Center, San Antonio, in 1991 and did postdoctoral training with Tom Schwan at NIAID's Rocky Mountain Laboratories (RML) in Hamilton, Mont. In 2001, he joined the Laboratory of Human Bacterial Pathogenesis at RML as a tenure-track investigator. He is now a senior investigator and head of the Plague Section in the Laboratory of Zoonotic Pathogens at RML.*

Yersinia pestis, the gram-negative bacterial agent of bubonic and pneumonic plague, is one of the most invasive and virulent bacterial pathogens, and human plague remains an international public health concern. *Y. pestis* also has recognized potential as an agent of bioterrorism. There is currently no vaccine available for plague, and immune correlates of protection against plague are incompletely characterized. New diagnostic tests are also needed.

We study the molecular mechanisms of plague transmission, infection, and immunity.

Y. pestis is a zoonotic pathogen that primarily infects wild rodents and is transmitted by fleas. Using the rat flea *Xenopsylla cheopis* (the primary vector of plague to humans in most parts of the world), we have identified several *Y. pestis* genes that are required to produce a transmissible infection in the flea, and we have characterized the bacterial "transmission phenotype."

We have also established mouse and rat models to characterize the bacterial and host response during the progression of bubonic plague. Microarray analyses of *Y. pestis* and rat gene expression in the infected lymph node (bubo) as well as immunologic analyses support a model in which the fulminant nature of plague depends on the

expression of several *Y. pestis* virulence factors that thwart the mammalian innate immune response. We are interested in understanding the in vivo function of these virulence factors and determining their specific targets and mechanisms of action.

Another goal is to expand and use these model systems to identify and evaluate new *Y. pestis* antigens for use in plague vaccines and diagnostics and to characterize the host response to naturally acquired infection. In collaboration with extramural researchers, we have already used our small-animal models to assess the efficacy of second-generation plague vaccines and delivery systems.

As one of the only laboratories in the world capable of studying plague transmission by fleas, we are also developing models to examine the vector-bacteria-host transmission interface and specific factors in the dermal fleabite site, such as flea saliva, that affect nascent infection and immunity.

Olivia Steele-Mortimer *received her Ph.D. in cell biology from the University of London in 1994 for work carried out at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. She did her postdoctoral training with B. Brett Finlay at the University of British Columbia in Vancouver, Canada, and with Philip Stahl at Washington University, St. Louis. She became a tenure-track investigator at the Rocky Mountain Laboratories, NIAID, in 2001. She is currently a senior investigator, leading the Salmonella-Host Cells Interactions Section in the Laboratory of Intracellular Parasites.*

The research in my laboratory focuses on the interaction of Salmonella with mammalian host cells. The genus Salmonella consists of more than 2,000 serovars that differ widely in host range and ability to cause disease despite the fact that they are almost identical genetically. The serovar that we work with is Typhimurium, one of the most common causes of bacterial gastroenteritis in the developed world.

My interest in host pathogen interactions began during my undergraduate days at the University of London, where I studied microbiology. However, it was at the EMBL cell biology department that I really started to appreciate the level of complexity involved in pathogen–host cell interactions. At that time cellular microbiology was just emerging as a new field and there was an apparent smorgasbord of pathogens about which very little was known.

Olivia Steele-Mortimer

I chose to work on Salmonella because this facultative intracellular pathogen can be genetically manipulated. My initial project proposal was to characterize the Salmonella-containing vacuole (SCV), a modified phagosome within which the intracellular bacteria survives and replicates. This has proven to be more of a challenge than we expected, but I was very lucky to start working on it just as light microscopy moved into the age of digital and live-cell imaging. We are now using a spinning-disc confocal microscope to define SCV dynamics and biogenesis in living cells. One of our major findings is that SCV biogenesis involves continuous dynamic interactions with the endocytic pathway and includes direct fusion with terminal lysosomes.

Like many other Gram-negative pathogens, Salmonella serovars inject bacterial effector proteins directly into host cells using specialized type III secretion systems (T3SS). We are interested in identifying these effector proteins and their targets in the host cell. One of the effectors we study, SopB/SigD, is an inositol phosphatase that causes activation of the pro-survival kinase Akt/PKB in Salmonella-infected epithelial cells. How this signal occurs is still not understood, but it seems to be an important anti-apoptotic signal that may be required in the establishment of infection in the intestinal epithelium.

Richard T. Wyatt

Most recently we have also started investigating the expression of Salmonella virulence genes using a combination of microar-rays, for transcriptome analysis, and GFP transcriptional fusion, for the detection of gene expression in individual bacteria. Altogether our work should reveal new aspects of pathogen–host cell interactions while also providing insights into basic host-cell processes such as membrane trafficking and signal-transduction pathways.

Richard T. Wyatt *received his Ph.D. in immunology in 1991 from the Sackler School of Biomedical Sciences at Tufts University School of Medicine in Boston. He came to NIAID in 2001 after serving as a research fellow and a junior faculty member at both the Dana-Farber Cancer Institute and Harvard Medical School in Boston. Since that time, he has headed the Vaccine Research Center's Structural Virology Section as chief and now senior investigator.*

The major focus of my laboratory is to understand the immunologic, structural, biochemical, biophysical, and antigenic properties of the HIV envelope glycoproteins, gp120 and gp41.

My goal is to translate this information into the rational design of immunogens to better elicit broadly neutralizing antibodies and to understand the mechanisms of broad neutralization accomplished via natural infection.

Such studies may also reveal novel structure-function relationships to better elucidate mechanisms of immune evasion or viral entry.

I plan to use the foundations established by structure, biochemistry, and neutralizing antibody mapping analysis to build a systematic process of HIV immunogen design.

This process includes protein design and protein evaluation through in vivo immunogenicity studies, mapping the elicited immune responses to feedback, once again upon rational immunogen modifications.

The end goal of eliciting neutralizing antibodies by rational immunogen design will contribute toward the VRC mission to develop a safe and effective HIV vaccine. Integral to this process is a close interaction with other VRC investigators that has led to published studies on gp120 in complex with CD4, the neutralizing antibodies b12 and 2F5, and the identification of broadly neutralizing antibodies in relatively rare HIV-infected individuals.

The following are the specific aims of the research in my lab:

- To define the HIV-1 envelope glycoprotein–neutralizing determinants targeted in broadly neutralizing patient sera
- To determine relevant and novel HIV envelope glycoprotein conformations by biochemical and structural analysis
- To integrate the foundations established by biochemical, biophysical, immunological, and structural information to rationally design HIV-1 immunogens capable of more efficiently generating neutralizing antibodies ■

Catalytic Reactions?

If you have a photo or other graphic that reflects an aspect of life at NIH (including laboratory life) or a quotation that scientists might appreciate that would be fit to print in the space to the right, why not **send it to us via e-mail: catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2E26.**

Also, we welcome letters to the editor for publication and your reactions to anything on the *Catalyst* pages.

In Future Issues...

- Research Festival Highlights
- Embedded Chemistry Librarians
- Biomarkers

Kids' Catalyst: Movement in Water

Hot, cold, carbonated, or caffeinated, water is the primary (if not the only) ingredient in what we drink every day. Let's see how this abundant and precious liquid responds when some (easily observed) substance is dropped into it—and let's see whether there's a difference if the water is still or stirred.

Celia Hooper

Somewhere off the coast of Maine

Goodies to Gather

- Six clear plastic cups, all the same size, and half-filled with water
- Food coloring
- Time-keeping device
- Paper and pencil to record our findings
- Stirring device

First, let's start with still water. Add a drop of food coloring and, using a timer, see how long it takes for the whole glass to turn a light shade of that color. You'll see a ripple here, a ripple there, and a ball of color reaching out to the rest of the glass. What do you think the difference will be when you drop the coloring in from a greater distance from the surface of the water? (Be careful when you do this, though, unless you want a splashy experimental fashion statement.)

Do you think that doing the same thing with hot or cold water will make any difference?

Next, the big stir: slowly stir a glass full of water, remove the stirring device, and then add the food coloring. You will now really have to pay attention to the time because the water will turn colors very quickly. What if you stir it just a little bit? Time the difference and see. I think you'll be surprised with the results!

If you have gallon or half-gallon clear plastic (or glass) bottles, you might try the same experiment. It will take longer, of course, but it will also be easier to see the progress, particularly with the stirred water.

So now you have seen how, with the least provocation, a single drop of color will quickly spread throughout a whole container of water. How quickly do you think it would take for gallons of a substance dumped daily into a lake, say, or an ocean to spread throughout that body of water?

—Jennifer White

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 2, Room 2E26, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

Publisher
Michael Gottesman
Deputy Director
for Intramural Research, OD

Editors
John I. Gallin
Director, NIH Clinical Center

Henry Metzger
Scientist Emeritus

Managing Editor
Fran Pollner

Writer-Editor
Christopher Wanjek
Director of Communications, OIR

Copy Editor
Shauna Roberts

Contributing Writers
Julie Wallace
Jennifer White

U.S. Department of Health and Human Services
National Institutes of Health
Building 2, Room 2E26
MSC 0235
Bethesda Maryland 20892

FIRST-CLASS MAIL
POSTAGE & FEES PAID
DHHS/NIH
Permit No. G-802
Publication No. 07-6250

Official Business
Penalty for Private Use $300

Printed on 50% recycled content paper and can be recycled as office white paper.